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SEQUENCE LISTING

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Helliwell, Christopher Andrew
Waterhouse, Peter Michael

<120> Efficient gene silencing in plants using short dsRNA sequences

<130> 021565-156

<140> US 10/780,638
<141> 2004-02-19

<150> US 60/447,711
<151> 2003-02-19

<160> 33

<170> PatentIn version 3.1

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Arabidopsis thaliana var. Landsberg erecta

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tctcttcttc tatcttaaat ctgtgtatac tatttcacta ttgggctttt tattagtcta    180
taatgggact caaaataagg ctttggccca catcaaaaag ataagtcaca aatcaaaaact    240
aaattcagag tcttttctcc cacatcggtc actgtactca ttttgtgttt gtttatatat    300
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        of the transcription initiation site.

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<223> XhoI restriction site

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<223> poly T stretch

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ctctcttctt ctatcttaaa tctgtgtata ctatttcact attgggcttt ttattagtct    180
ataatgggac tcaaaataag gctttggccc acatcaaaaa gataagtcac aaatcaaaac    240
taaattcaga gtcttttctc ccacatcggt cactgtactc ttttgtgttt gtttatatat    300
tacacgaacc gatctttggt acgtcgagct aagtaacatg agcttgtaac ccatgtgggg    360
acattaagat ggtggaacac tggctcgggt ccacggggcg gttctgttgt cgactttttt    420
tttctcgag                                     429

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 var. Landsberg erecta

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 <222> (314)..(319)
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 <223> poly T stretch

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gaattcttat gcagcctgtg atggataact gaatcaaaca aatggcgtct gggtttaaga	60
agatctgttt tggctatggt ggacgaaaca agtgaacttt taggatcaac ttcagtttat	120
atatggagct tatatcgagc aataagataa gtgggctttt tatgtaattt aatgggctat	180
cgtccataga ttcactaata cccatgccca gtacccatgt atgcgtttca tataagctcc	240
taattttctcc cacatcgctc aaatctaaac aaatcttggt gtatatataa cactgagga	300
gcaacattgg tcacgatcgt ttttttttga attc	334

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 thaliana var. Landsberg erecta including 136 bases downstream
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 <223> Pol III promoter region

<220>

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 <223> XhoI restriction site

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 <222> (453)..(461)
 <223> poly T stretch

<220>
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 <222> (462)..(467)
 <223> EcoRI restriction site

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gaattcttat	gcagcctgtg	atggataact	gaatcaaaca	aatggcgtct	gggtttaaga	60
agatctgttt	tggtatgtt	ggacgaaaca	agtgaacttt	taggatcaac	ttcagtttat	120
atatggagct	tatatcgagc	aataagataa	gtgggctttt	tatgtaattt	aatgggctat	180
cgtccataga	ttcactaata	cccatgcccc	gtacccatgt	atgcgtttca	tataagctcc	240
taattttctc	cacatcgctc	aaatctaaac	aaatcttggt	gtatatataa	caactgagga	300
gcaacattgg	tcacgacctt	acttgaacag	gatctgttct	ataggctcgt	acctctgttt	360
ccttgatttc	tcaagagaca	ggcccttaac	cctggttgat	gaaccatgac	cgtgaggcta	420
gagcgtgatt	gacggctacg	atcgtcctcg	agtttttttt	tgaattc		467

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<220>
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 <223> XhoI restriction site

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 <223> Pol III promoter region

<220>
 <221> misc_feature
 <222> (437)..(442)
 <223> SalI restriction site

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 <223> poly T stretch

<220>
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<400> 5

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cttgaaccgt agctttcgtt ttcttctttt taactttcca ttcggagttt ttgtatcttg      180
tttcatagtt tgtcccagga ttagaatgat taggcacgca accttcaaga atttgattga      240
ataaaacatc ttcatcttta agatatgaag ataactttca aaaggcccct gggaatctga      300
aagaagagaa gcaggcccat ttatatggga aagaacaata gtatttctta tataggccca      360
tttaagttga aaacaatctt caaaagtccc acatcgctta gataagaaaa cgaagctgag      420
tttatataca gctagagtcg actttttttt gagctc                                456

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<210> 6

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<220>

<221> misc_feature

<222> (7)..(468)

<223> Pol III promoter region

<220>

<221> misc_feature

<222> (469)..(474)

<223> PvuI restriction site

<220>

<221> misc_feature

<222> (475)..(482)

<223> Poly T stretch

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<221> misc_feature

<222> (483)..(488)

<223> XhoI restriction site

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ttagcttttt ttcttcttct tcgttcatac agtttttttt tgtttatcag cttacatttt      120
cttgaaccgt agctttcgtt ttcttctttt taactttcca ttcggagttt ttgtatcttg      180
tttcatagtt tgtcccagga ttagaatgat taggcacgca accttcaaga atttgattga      240
ataaaacatc ttcatcttta agatatgaag ataactttca aaaggcccct gggaatctga      300
aagaagagaa gcaggcccat ttatatggga aagaacaata gtatttctta tataggccca      360
tttaagttga aaacaatctt caaaagtccc acatcgctta gataagaaaa cgaagctgag      420
tttatataca gctagagtcg aagtagtgat tgtcccttcg gggacatccg atcggttttt      480
ttctcgag                                488

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<210> 7
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<220>
 <221> misc_feature
 <222> (7)..(384)
 <223> Pol III promoter region

<220>
 <221> misc_feature
 <222> (385)..(390)
 <223> PvuI restriction site

<220>
 <221> misc_feature
 <222> (391)..(399)
 <223> poly T stretch

<220>
 <221> misc_feature
 <222> (400)..(405)
 <223> EcoRI restriction site

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gaattcaagg gatctttaaa catacgaaca gatcacttaa agttcttctg aagcaactta	60
aagttatcag gcatgcatgg atcttggagg aatcagatgt gcagtcaggg accatagcac	120
aggacaggcg tcttctactg gtgctaccag caaatgctgg aagccgggaa cactgggtac	180
gttggaacc acgtgatgtg gagtaagata aactgtagga gaaaagcatt tcgtagtggg	240
ccatgaagcc ttccaggaca tgtattgcag tatgggccgg cccattacgc aattggacga	300
caacaaagac tagtattagt accacctcgg ctatccacat agatcaaagc tggtttaaaa	360
gagttgtgca gatgatccgt ggcacgatcg tttttttttg aattc	405

<210> 8
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 <223> EcoRI restriction site

<220>
 <221> misc_feature
 <222> (7)..(421)
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<220>
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 <222> (422)..(427)
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<220>
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<220>
 <221> misc_feature
 <222> (437)..(442)
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gaattctgag	agcatttgt	ggcgttctc	tgaattactt	actgtcactt	tgattggagc	60
cattattttc	agactctact	gaagattgaa	ttgaatgaga	aactatgaaa	ctttacaagt	120
gaattattat	ggagttcatg	gcaactgcta	tgaggttttt	cctactggga	attggaacgg	180
tttctacgaa	attaactgtc	cacacgttaa	aaatataaat	taatgcgtaa	ttgttatttt	240
ttctataaca	aataaaaaaac	tgaaatacga	cataaatttt	attactttta	ttgcacttta	300
gccttagaga	tattgcgttg	tagtcggcgt	aggtgtgtca	ggggccaata	tattgttccc	360
acatcggcag	tcgagcacat	aaactctagc	gttataagaa	tctatccact	atcaacggtc	420
acgatcgttt	ttttttgaat	tc				442

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 <222> (6)..(11)
 <223> PvuI restriction site

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<220>
 <221> misc_feature
 <222> (101)..(195)

<223> spacer sequence

<220>

<221> misc_feature

<222> (190)..(195)

<223> BamHI restriction site

<220>

<221> misc_feature

<222> (196)..(284)

<223> GUS sequence (antisense)

<220>

<221> misc_feature

<222> (285)..(290)

<223> PvuI restriction site

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<221> misc_feature

<222> (290)..(295)

<223> SalI restriction site

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gtcgacgac	gcagcgtaat	gctctacacc	acgccgaaca	cctgggtgga	cgatatcacc	60
gtggtgacgc	atgtcgcgca	agactgtaac	cacgcgtctg	ttgactggca	ggtggtggcc	120
aatggtgatg	tcagcgttga	actgcgtgat	gcggatcaac	aggtggttgc	aactggacaa	180
ggcactagcg	ggatccagac	gcgtgggttac	agtcttgccg	gacatgcgtc	accacggtga	240
tatcgtccac	ccaggtgttc	ggcgtggtgt	agagcattac	gctgcgatcg	tcgac	295

<210> 10

<211> 93

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 41 bp for silencing expression of the GUS gene (GUShp41)

<220>

<221> misc_feature

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<223> SalI restriction site

<220>

<221> misc_feature

<222> (7)..(42)

<223> GUS sequence (sense)

<220>

<221> misc_feature

<222> (43)..(51)

<223> spacer sequence

<220>

<221> misc_feature

<222> (52)..(87)

<223> GUS sequence (antisense)

<220>
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 <222> (88)..(93)
 <223> Sal I restriction site
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gtcgactggg cagatgaaca tggcatcgtg gtgattgatg aatgcgagaa cttcatcaat 60
 caccacgatg ccatgttcat ctgcccagtc gac 93

<210> 11
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 expression of the GUS gene (GUShp21)

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 <223> GUS sequence (sense)

<220>
 <221> misc_feature
 <222> (23)..(28)
 <223> spacer sequence

<220>
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 <222> (29)..(44)
 <223> GUS sequence (antisense)

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 expression of the PHYB gene, derived from the 5' end of PHYB
 (PHYB5hp 42)-upper strand

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accgccacgg ccaccgccac taccgccgac tccg 94
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<211> 94

<212> DNA

<213> Artificial Sequence

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tcgacggagt cgggggtagt ggcgggtggcc gtggcggtgg ccgtggcctc ctccacggcc 60
accgccacgg ccaccgccac taccgccgac tccg 94
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<210> 14

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<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 21)-upper strand

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tcgacggagt cgggggtagt ggcgggaggag gccgccacta ccccgactc cg 52
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<210> 15

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 21)-lower strand

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tcgacggagt cgggggtagt ggcgggcctcc tccgccacta ccccgactc cg 52
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<210> 16

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 42)-upper strand

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gccatatccc tacatggctg aaccacacca tcca 94
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<211> 94

<212> DNA

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tcgatggatg gtgtgggttca gccatgtagg gatatggcgg gggaaccctc ctgttccccc 60
gccatatccc tacatggctg aaccacacca tcca 94
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<212> DNA

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<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing

expression of the PHYB gene, derived from the 3' end of PHYB
(PHYB3hp 42)-upper strand

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tcgacattgt caactgctag tggaagtggg gacatgatgc tgatgaagga ggtcatcagc 60
atcatgtcac cacttcact agcagttgac aatg 94

<210> 21

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing
expression of the PHYB gene, derived from the 3' end of PHYB
(PHYB3hp 42)-lower strand

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atcatgtcac cacttcact agcagttgac aatg 94

<210> 22

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<220>

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<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing
expression of the PHYB gene, derived from the 3' end of PHYB
(PHYB3hp 21)-lower strand

<400> 23

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<210> 24

<211> 94

<212> DNA

<213> Artificial Sequence

<220>
 <223> sequence of the dsRNA encoding region of 42 bp for silencing
 expression of the PDS gene (PDS42)-upper strand

<400> 24

tcgacttaac ttgtaaggaa tattacgata ctaaccgggc aatgctagga ggagcattga 60
 ccggtagga tcgtaatat cttacaagt taag 94

<210> 25
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<220>
 <223> sequence of the dsRNA encoding region of 42 bp for silencing
 expression of the PDS gene (PDS42)-lower strand

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tcgacttaac ttgtaaggaa tattacgata ctaaccgggc aatgctcctc ctagcattga 60
 ccggtagga tcgtaatat cttacaagt taag 94

<210> 26
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 <212> DNA
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<220>
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 expression of the PDS gene (PDS21)-upper strand

<400> 26

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<210> 27
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<220>
 <223> sequence of the dsRNA encoding region of 21 bp for silencing
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<400> 27

tcgacttaac ttgtaaggaa tattacctcc ttaatatcc ttacaagta ag 52

<210> 28
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 <223> small hairpin RNA coding region (GUS_A)

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 <222> (12)..(53)
 <223> sense RNA encoding region

<220>
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 <222> (54)..(62)
 <223> loop structure

<220>
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 <222> (63)..(104)
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<400> 28

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gtcgacgatac gtgcggtcac tcattacggc aaagtgtggg tcaataatca ggagttcctt    60
cttcctgatt attgaccac actttgccgt aatgagtgac cgcagtcgac gatcg      115
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 <223> small hairpin RNA coding region (GUS_B)

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 <222> (9)..(50)
 <223> sense RNA encoding sequence

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 <222> (51)..(59)
 <223> loop structure

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<221> misc_feature
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 <223> antisense RNA coding region

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 <222> (102)..(112)
 <223> SalI/pvuI restriction site

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gtcgacgacg gtcacgaaga tgcggacttg cgtggcaaag gattcgataa gttccttctt    60
tatcgaatcc ttgcccacgc aagtcgcat cttcatgacg agtcgacgat cg              112
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<210> 30
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<220>
 <223> small hairpin RNA coding region (GUS_C)

<220>
 <221> misc_feature
 <222> (1)..(11)
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 <222> (12)..(53)
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 <222> (54)..(62)
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<220>
 <221> misc_feature
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 <223> SalI/PvuI restriction sites

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gtcgacgacg gtgcgacctc gcaaggcata ttgcgcgttg gcggtacaaa gaagttcctt    60
ctttcttggt accgccaacg cgcaatatgc cttgcgaggt cgagtcgac gatcg          115
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